

FIG. 1A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS				1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
		1.00E+03	5.00E+03	1.00E+04	5.00E+04							
Thr	X-X-X-X-Thr-X-X-X-X-X-X-X-X-X-X	1.02	1.32	0.84	0.34	0.07	0.04	0.02				
Ser	X-X-X-X-X-Ser-Thr-X-X-X-X-X-X-X-X-X-X	0.11	0.05	0.01	0.00	0.01	0.00	0.00				
Threonine* mix	18 phospho-Thr peptide	1.84	1.13	0.40	0.28	0.10	0.07	0.05				
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01	0.00				
AK1-Thr308-P	Ile-Lys-Asp-Gly-Ala-Thr-Met-Lys-Thr-Phe-Gly-Gly-Thr-Pro	1.18	0.85	0.24	0.13	0.03	0.01	0.00				
APPI-Thr688-P	Asp-Ala-Met-Thr-Phe-Lys-Lys-Lys-His-Leu-Ser-Lys-Gly	0.14	0.03	0.01	0.01	0.01	0.01	0.01				
C3-P	Asp-Thr-Gln-Ile-Lys-Arg-Thr-Phe-Val-Gly-Thr-Pro-Phe-Gly	1.71	1.13	0.39	0.22	0.03	0.02	0.02				
CAK-Thr167-P	His-Gln-Val-Val-Thr-Arg-Trp-Tyr-Arg-Gly	1.77	1.15	0.41	0.27	0.08	0.03	0.01				
CAMIV-Thr198-P	His-Gln-Val-Val-Thr-Arg-Trp-Tyr-Arg-Gly	1.79	1.38	0.83	0.40	0.09	0.05	0.01				
ODC ² -Thr167-P	Ile-Pro-Gln-Val-Thr-Thr-His-Leu-Val-Thr-Leu-Gly	1.02	0.58	0.14	0.08	0.03	0.01	0.01				
CDK2-Thr159-P	Gly-Val-Phe-Val-Thr-Thr-His-Leu-Val-Thr-Leu-Gly	1.88	1.70	0.51	0.24	0.08	0.04	0.02				
p70S6K-Thr389-P	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Gly	1.08	1.44	0.82	0.30	0.05	0.04	0.01				
PKCalpha-P	Lys-Glu-His-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Gly	1.82	1.83	0.94	0.38	0.15	0.08	0.02				
ERK2-P	Asp-His-Thr-Gly-Phe-Leu-Thr-Phe-Thr-Val-Ala-Met-Arg-Trp-Gly	1.58	1.18	0.51	0.30	0.07	0.04	0.02				
Myc Ser58/62-P	Glu-Leu-Leu-Pro-Thr-Phe-Pro-Leu-Ser-Phe-Ser-Arg-Ser-Gly	0.11	0.05	0.03	0.02	0.02	0.02	0.02				
P39-2P	Leu-Ala-Arg-His-Thr-Asp-Lys-Glu-Met-Thr-Gly-Tyr-Val-Ala-Thr-Ala-Gly	0.84	0.30	0.08	0.03	0.01	0.04	0.02				
JNK-2P	Ser-Phe-Met-Met-Thr-Pro-Tyr-Val-Val-Thr-Arg-Tyr-Arg-Gly	1.49	0.44	0.12	0.07	0.03	0.02	0.02				

(SEQ ID NO 13)

FIG. 1B

PEPTIDE SEQUENCE	phospho-Thr Reactivity
XXXXXXS*XXXXXX	—
XXXXY*XXXX	—
XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	++
XXXXBSXS*XPXXXX (SEQ ID NO:15)	—
XXXXBSXS*XPXXXX (SEQ ID NO:16)	—
XXXXXPXS*/T*PXXXXX (SEQ ID NO:17)	++
XXXXXPXS/TPXXXXX (SEQ ID NO:18)	—
XXXXXT*XXXXXX	+++
XXXXXXS/TXXXXXX	—
21 phospho-Thr peptides mixture	+++
38 phospho-Ser peptides mixture	—
30 phospho-Tyr peptides mixture	—
NEB LIBRARY	
X-X-X-X-D/E-X-X-S*/T*-X-X-X-X-X-C (SEQ ID NO:19)	+++
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	++
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+++
X-X-X-X-R/K-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+++
X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:23)	+++
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+++
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-C (SEQ ID NO:25)	+++
X-X-X-X-X-X-S*/T*-P-X-X-X-X-X-X-C (SEQ ID NO:26)	++
X-X-X-X-X-T*-X-X-X-X-X-X-C	+++
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-X-C (SEQ ID NO:27)	++
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-X-C (SEQ ID NO:29)	++
ANTIBODY REACTIVITY	
+++ very strong	ELISA O.D. > 2
++ strong	1 - 2
+ weak	0.2 - 1
— very little	< 0.2

FIG. 1C

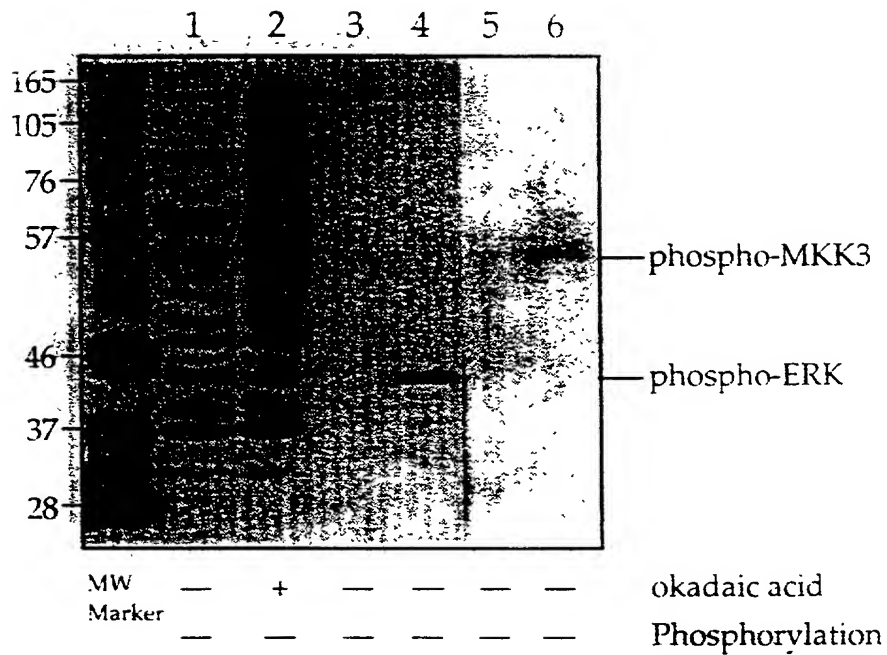


FIG. 1D

Fixed Amino Acid	-5-4-3-2-1 X X X X Ser*Thr* X X X X X Fixed AA position relative to phospho-Ser*/Thr*							+1+2+3+4+5		
	-4	-3	-2	-1	S*/T*	+1	+2	+3		
Ala	+	+	+	+		+	+	+		
Cys	+	+	+	+		+	+	+		
Asp	+	+	+	+		+	+	+		
Glu	+	+	+	+		+	+	+		
Phe	+	+	+	+		+	+	+		
Gly	+	+	+	+		+	+	+		
His	+	+	+	+		+	+	+		
Ile	+	+	+	+		+	+	+		
Lys	+	+	+	+		+	+	+		
Leu	+	+	+	+		+	+	+		
Met	+	+	+	+		+	+	+		
Asn	+	+	+	+		+	+	+		
Pro	+	+	+	+		+	+	+		
Gln	+	+	+	+		+	+	+		
Arg	+	+	+	+		+	+	+		
Ser	+	+	+	+		+	+	+		
Thr	+	+	+	+		+	+	+		
Val	+	+	+	+		+	+	+		
Trp	+	+	+	+		+	+	+		
Tyr	+	+	+	+		+	+	+		

FIG. 2A

PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
PSAP	X-X-X-X-X-Pro-X-Ser-Thr-Pro-X-X-X-X-Cys (SEQ ID NO: 27)	1.82	1.97	1.74	1.40	0.70	0.35	0.08
Threonine mix	18 phospho-Thr peptide mix	1.97	1.37	0.67	0.36	0.13	0.07	0.05
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-Cys (SEQ ID NO: 28)	0.14	0.03	0.01	0.00	0.00	0.00	0.00
RB Thr373-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Alg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30)	2.07	2.17	1.70	1.20	0.48	0.18	0.03
MRK3-Thr-P	Ser-Val-Ala-Lys-Thr-Met-Asp-Ala-Gly-Cys (SEQ ID NO: 31)	0.06	0.04	0.01	0.00	0.00	0.00	0.00
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO: 32)	0.05	0.02	0.01	0.00	0.01	0.00	0.00
p70 S6K-Thr389	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO: 33)	0.11	0.06	0.01	0.00	0.01	0.00	0.00
cdk4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr-Pro-Val-Val-Val-Lys-Cys (SEQ ID NO: 32)	2.07	2.21	2.01	1.55	0.69	0.31	0.00

FIG. 2B

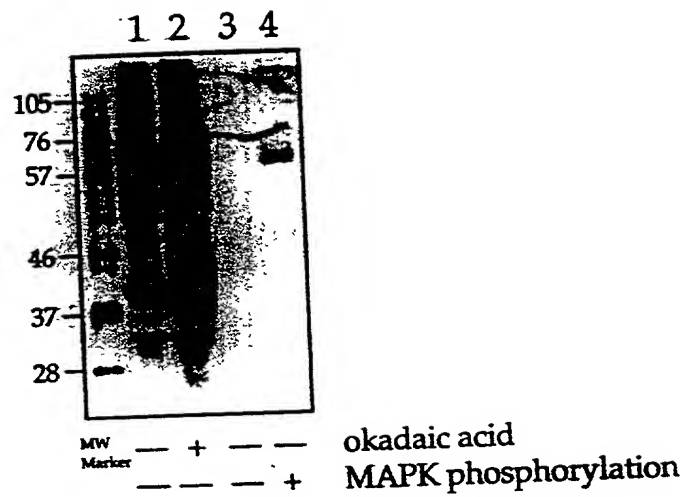


FIG. 3A

PEPTIDE	SEQUENCE	ANTIBODY DILUTIONS							
		1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05		
14-3-3 BM-P	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-Cys (SEQ ID NO: 33)	2.41	2.15	1.49	1.15	0.44	0.25		
14-3-3 BM	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-Cys (SEQ ID NO: 34)	0.07	0.03	0.02	0.03	0.02	0.03		
CDC25-Ser216-P	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys (SEQ ID NO: 35)	2.35	2.08	1.49	1.05	0.33	0.18		
CDC25-Ser216	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys (SEQ ID NO: 36)	0.05	0.02	0.03	0.03	0.04	0.03		
Bad-Ser112-P	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys (SEQ ID NO: 37)	1.59	0.43	0.10	0.03	0.01	0.00		
Bad-Ser112	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys (SEQ ID NO: 38)	0.00	0.00	0.00	0.00	0.00	0.00		
Bad-Ser136	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys (SEQ ID NO: 39)	0.03	0.00	0.00	0.00	0.00	0.00		
Bad-Ser136-P	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys (SEQ ID NO: 40)	3.25	1.86	0.73	0.51	0.07	0.03		

FIG. 3B

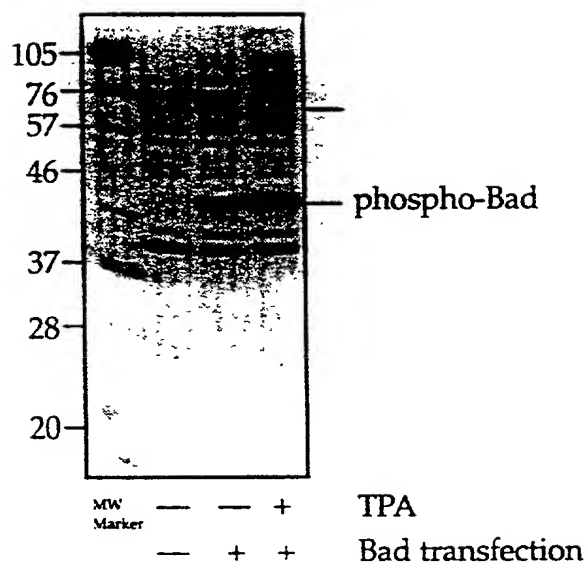


FIG. 4A

PEPTIDE	SEQUENCE	MONOCLONAL ANTIBODIES
		888 8A9
Ser/ThrPro-P	X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO: 26)	1.774 0.731
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO: 27)	0.924 0.766
ProXSer/ThrPro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO: 41)	0.02 0.083
ProXSer/ThrProXArg-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-Arg/Lys-X-X-X-Cys (SEQ ID NO: 42)	1.955 1.275
Thr-P	X-X-X-X-X-Thr-X-X-X-X-X-Cys	0 --
Ser-P	X-X-X-X-X-Ser-X-X-X-X-X-Cys	0.031 0.088
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-X-Cys	0.021 0.066
Tyr-P	X-X-X-X-X-Tyr-X-X-X-X-X-Cys	0.023 0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser-Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO: 43)	0.032 0.124
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30)	3.336 3.503
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 44)	0.02 0.073

FIG. 4B

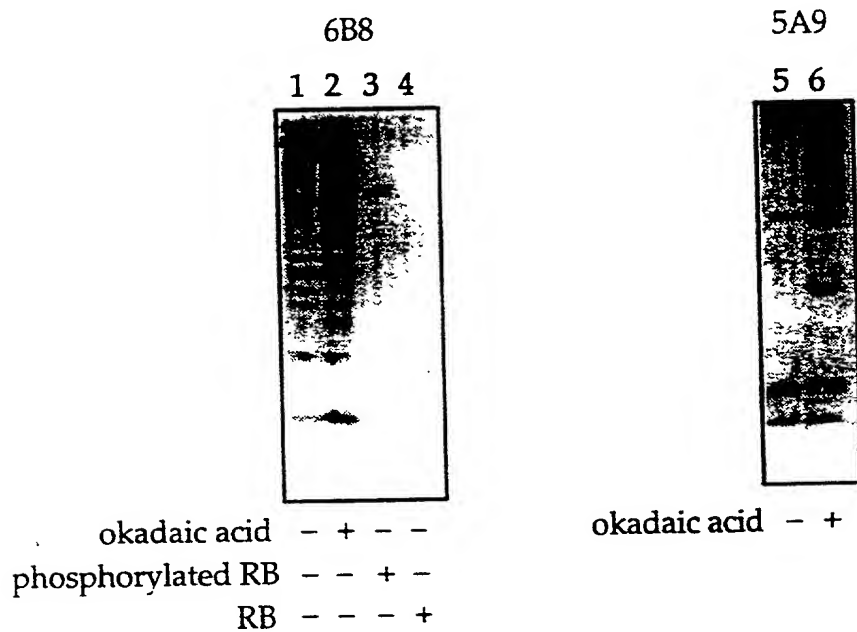


FIG. 5A

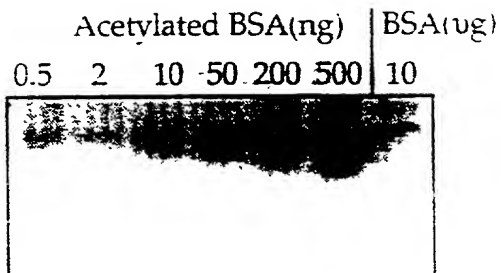


FIG. 5B



FIG. 5C



FIG. 5D



Phospho-Akt Substrate Antibody

Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens
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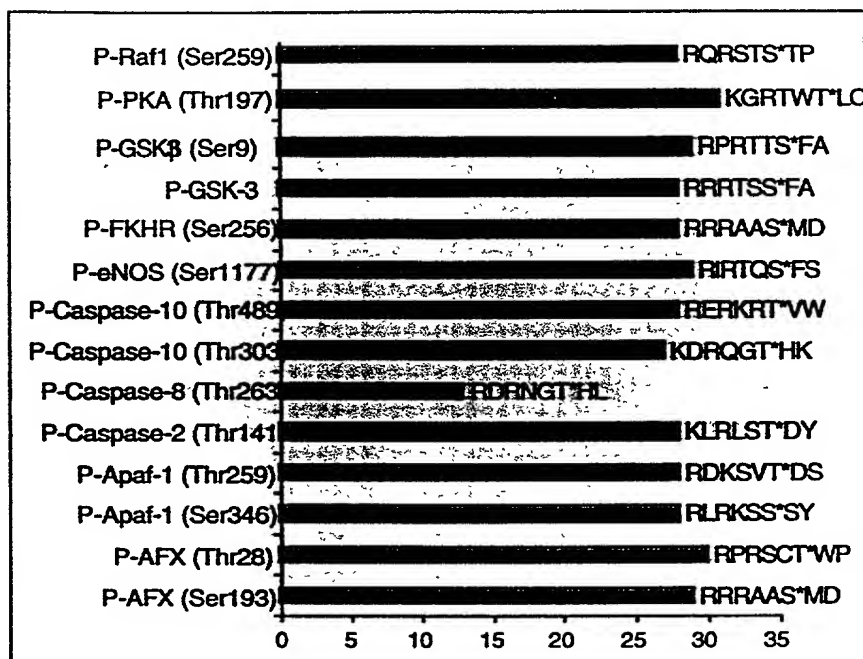
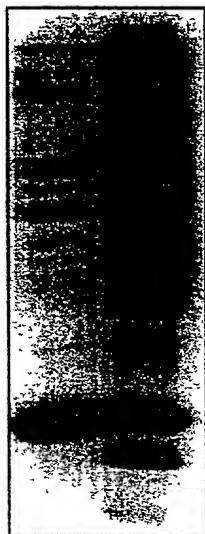


Figure 6: Signal to noise ratio of ELISA readings using Phospho-Akt Substrate Antibody with phospho-peptides of Akt substrates vs. non-phospho-peptides of Akt substrates.



- +
calyculin A

Figure 7: Western analysis of calyculin A-treated A431 cells using Phospho-Akt Substrate Antibody.

Phospho-PKA Substrate Antibody

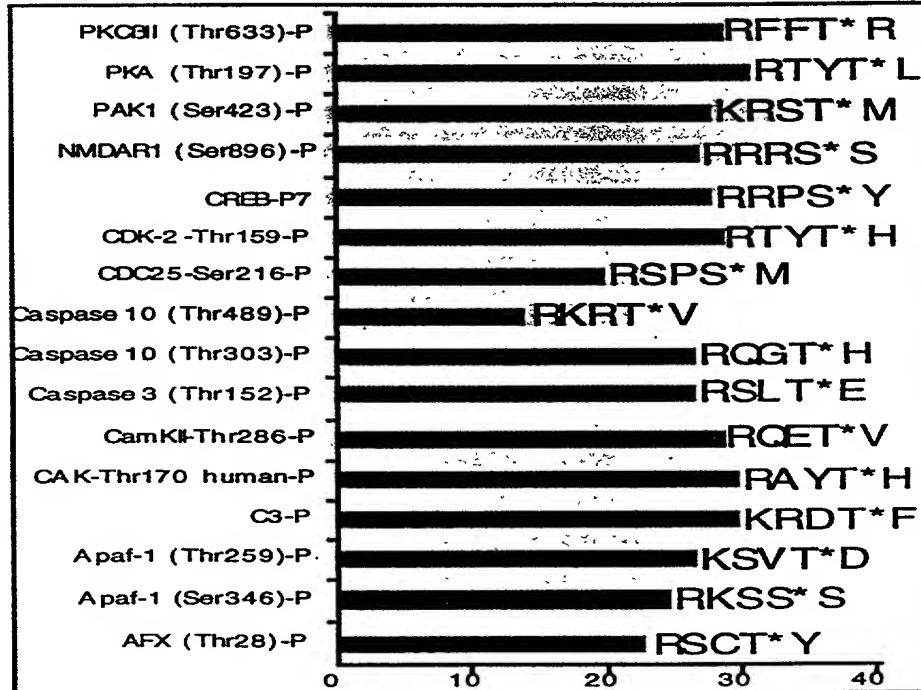
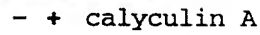
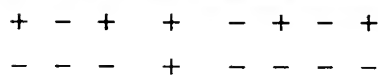


Figure 8: Signal to noise ratio of ELISA reading using phospho-PKA substrates antibody against peptides have Arginine or Lysine at -3 position.

[illegible]

Production of Motif-Specific Context-Independent Antibodies Using Peptide Libraries as Antigens
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Cell Extracts
PKI

Phospho-Serine/Threonine Phenylalanine Antibody

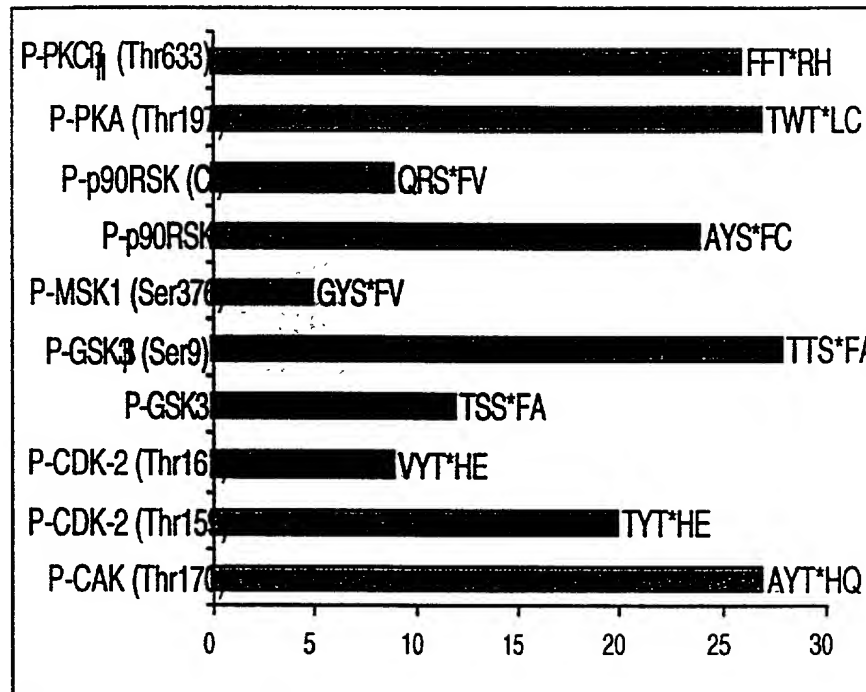


Figure 11: Signal to noise ratio of ELISA reading using phospho-Serine/threonine phenylalanine antibody against the peptides surrounded by phenylalanine, tyrosine or tryptophan.

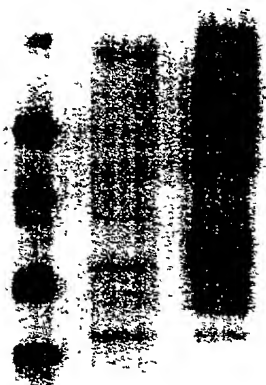


Figure 12: Western analysis of calyculin A-treated A431 cells using phospho-Serine/phenylalanine substates antibody.

- +
calyculin A

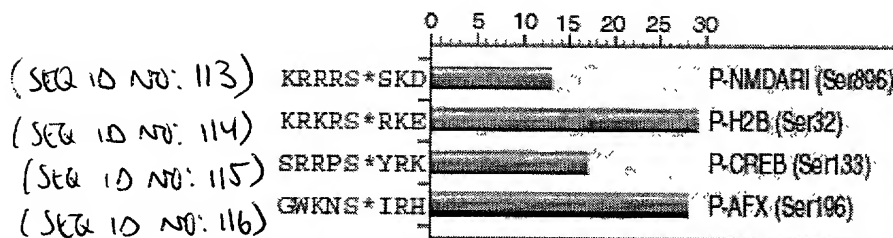


Figure 13. Signal to noise ratio of ELISA reading, using a context-independent antibody specific for the phospho-PKC consensus substrate motif, with phospho-PKC substrate containing peptides and nonphospho peptides.

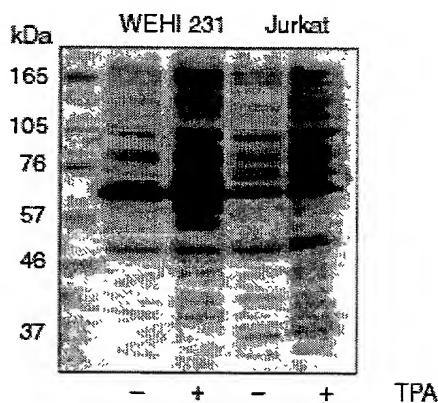


Figure 14. Western blot analysis of WEHI 231 cells or Jurkat cells untreated (-) or treated (+) with TPA, probed with a context-independent antibody specific for the phospho-PKC consensus substrate motif.

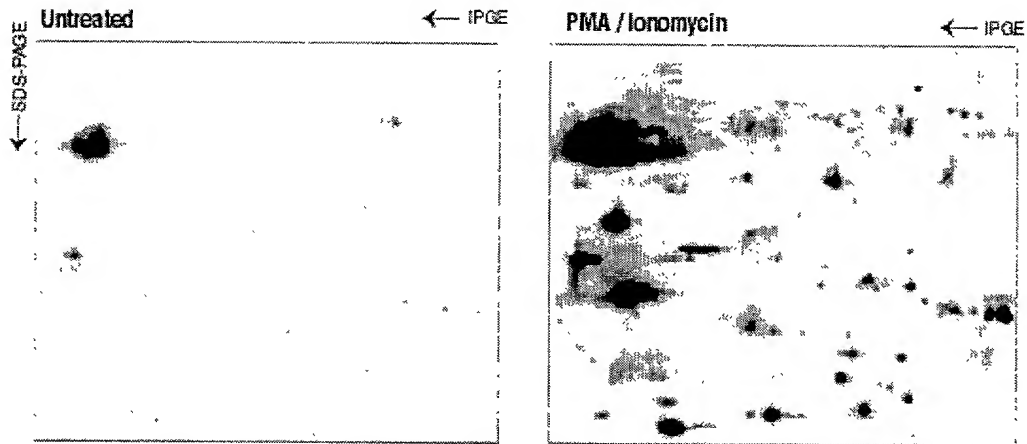


Figure 15. Western blot analysis of whole cell lysates of Jurkat cells untreated and treated with PMA (50 ng/ml) and ionomycin (1 μ M) for 20 minutes prior to lysis, using a context-independent antibody specific for the phospho-PKC consensus substrate motif. Proteins were separated by 2D electrophoresis prior to blotting.

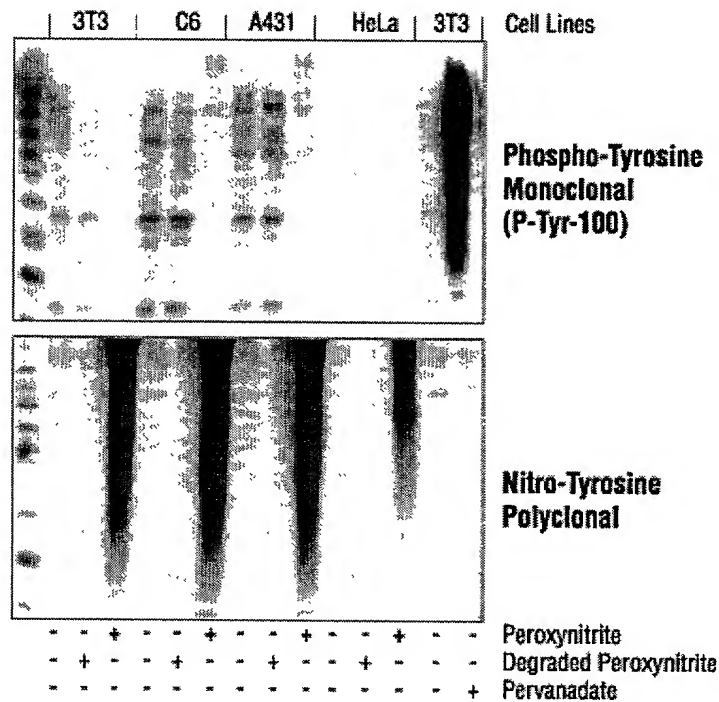


Figure 16. Western analysis of whole cell lysates of different cells untreated or treated with peroxynitrite, degraded peroxynitrite or pervanadate using a context-independent antibodies specific for phosphotyrosine (upper), and a polyclonal context-independent antibody specific for nitrotyrosine (lower).

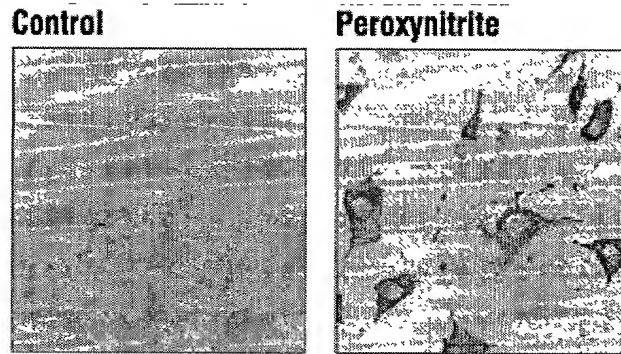


Figure 17. Immunocytochemical staining of NIH/3T3 cells treated with degraded peroxynitrite (control) or peroxynitrite using a polyclonal context-independent antibody specific for nitrotyrosine (brown).

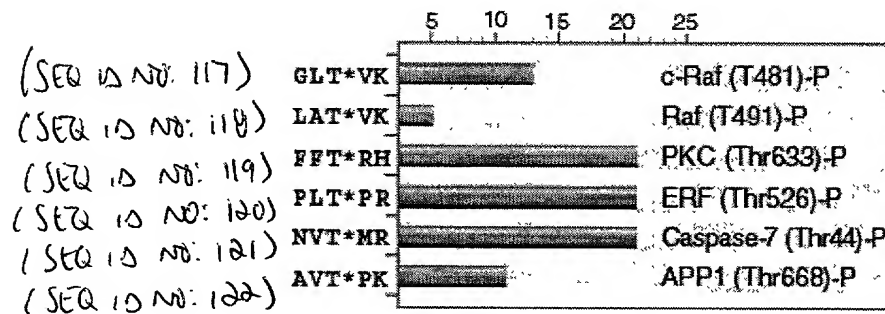


Figure 18. Phosphothreonine-X-arginine motif-specific context-independent antibody ELISAs: Signal to noise ratio of phospho versus nonphospho peptides containing the phospho-threonine-X-arginine motif. (T* denotes phosphorylated threonine.)

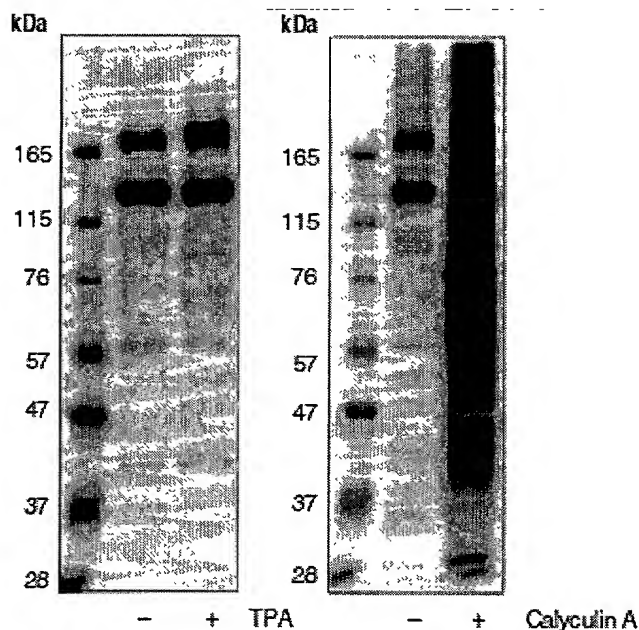


Figure 19. Western blot analysis of Jurkat cell extracts untreated (–) and treated (+) with TPA or Calyculin A, using a context-independent antibody specific for the phosphothreonine-X-arginine motif.

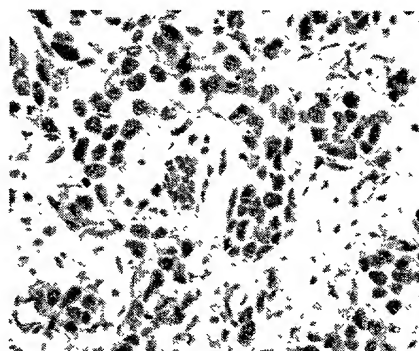


Figure 20. Immunohistochemical staining of proteins containing phosphorylated threonine-X-arginine motifs in paraffin-embedded human breast carcinoma, using a context-independent antibody specific for the motif.

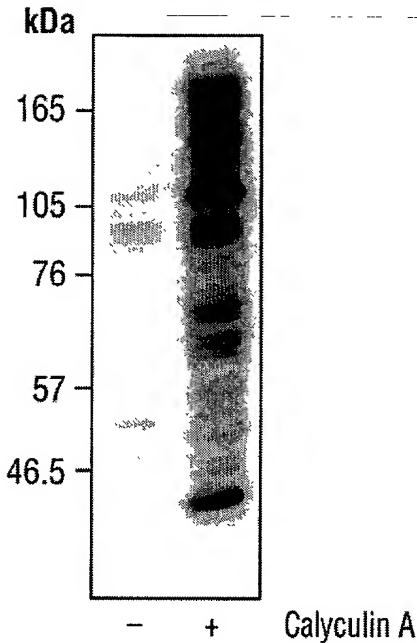


Figure 21. Western blot analysis of calyculin A treated A431 cells, using a context-independent antibody specific for the phospho-14-3-3 binding motif #2 (phospho(Ser)-Arg-X-(Tyr/Phe)-X-pSer).

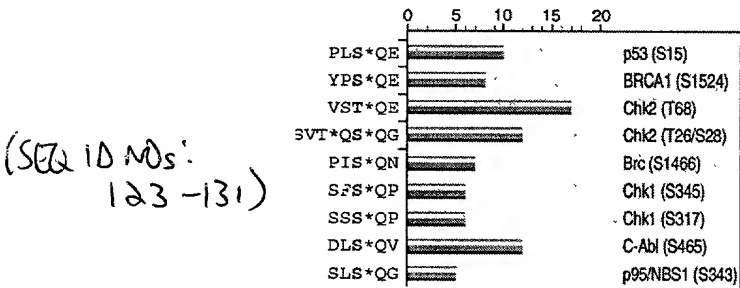


Figure 22. Phospho-ATM/ATR consensus substrate motif-specific, context-independent antibody ELISAs: Signal to noise ratio of phospho versus nonphospho peptides. (S* or T* denote phosphorylated serine or threonine.)

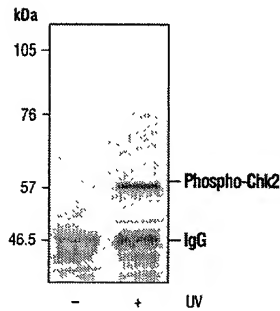


Figure 23. Chk2 transfected and UV treated COS cell extracts immunoprecipitated with Chk2 antibody then detected by Western blotting, using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.

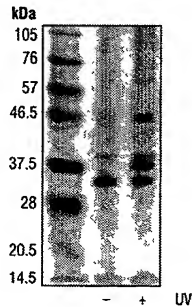


Figure 24. Western blot analysis of UV treated COS cells, using a context-independent antibody specific for phospho-ATM/ATR consensus substrate motif.

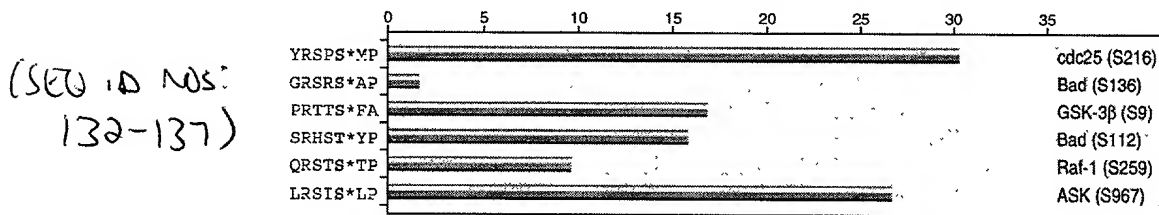


Figure 25. Phospho-14-3-3 binding motif-specific, context-independent monoclonal antibody ELISAs: Signal to noise ratio of phospho versus nonphospho 14-3-3 binding motif peptides. (T* and S* denote phosphorylated threonine and serine).

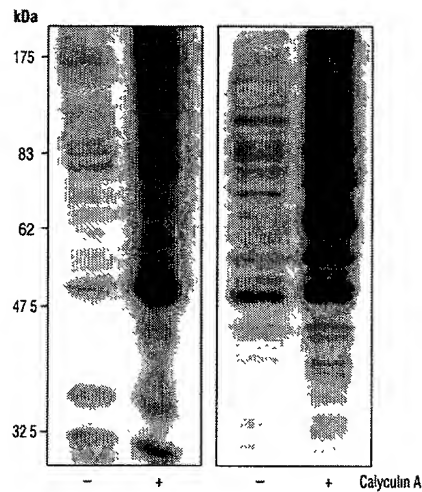


Figure 26. Western blot analysis of calyculin A treated A431 cells, using a context-independent antibodies specific for phospho-14-3-3 binding motif #1(left, monoclonal; right, polyclonal).

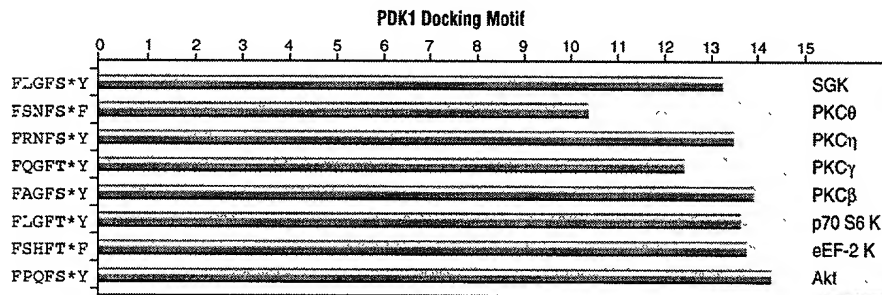


Figure 27. Phospho-PDK1 docking motif-specific, context-independent monoclonal antibody ELISAs: Signal to noise ratio of phospho versus non-phospho peptides corresponding to potential PDK1 docking motifs. (T* and S* denote phosphorylated threonine and serine.)

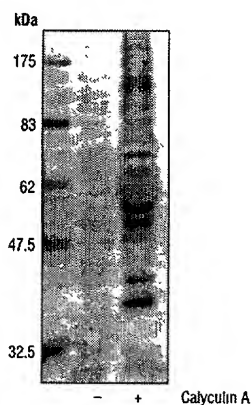


Figure 28. Western blot analysis of extracts from A431 cells untreated or treated with 0.1 μ M calyculin A for 30 minutes prior to lysis, using a monoclonal context-independent antibody specific for the phospho-PDK1 docking motif.

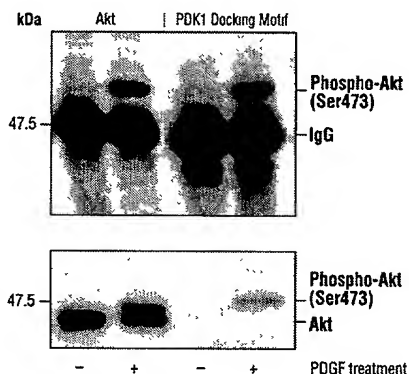


Figure 29. Immunoprecipitation of extracts from NIH/3T3 cells untreated or treated with 100 ng/ml of PDGF for 20 minutes prior to lysis, using a monoclonal context-independent antibody specific for phospho-PDK1 docking motif and an antibody specific for Akt, then probed with the PDK1 docking motif monoclonal antibody (upper) and the Akt antibody (lower).